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Hepatitis C Biochemical Remission and Viral Replication in Haemodialysis Patients

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The natural course of non-A, non-B (type C) hepatitis was studied in 62 haemodialysis patients. From the onset of the disease, serum alanine aminotransferase levels were monitored monthly for 9–218 mon (median 115). After fluctuation of aminotransferase levels for 1–206 mon (median 39), 57 (92%) patients showed normalization of these levels lasting until the end of the follow-up, which was for >2 yr in 31 (50%) cases and for >5 yr in 15 (24%) cases. At the end of follow-up, hepatitis C viraemia was assessed by reverse transcription-polymerase chain reaction (RT-PCR) and branched DNA (bDNA) assay. Viraemic levels were significantly lower in the 15 patients with normal aminotransferase for >5 yr (median RT-PCR+ve/bDNA-ve, range RT-PCR-ve to $10^{6.7}$ Eq/mL) than in the 47 cases with normal levels for <5 yr (median $10^{6.6}$, range RT-PCR+ve/bDNA-ve to $10^{7.6}$ Eq/mL) ($P < 0.01$). Moreover, a significant inverse relation was observed between viraemic levels and the duration of aminotransferase normalization ($r = -0.46$, $P < 0.01$). These findings indicate that biochemical remission of hepatitis C may be frequent in haemodialysis patients and may be related to viral attenuation.

KEY WORDS: chronic hepatitis C, hepatitis C virus antibody, hepatitis C virus RNA

INTRODUCTION

After the genome of hepatitis C virus (HCV) was cloned and screening assays for HCV antibodies had been developed [Choo et al., 1989; Kuo et al., 1989], HCV was shown to be the major causative agent of non-A, non-B hepatitis [Bruix et al., 1989; Colombo et al., 1989; Yuki et al., 1992a, b, c, 1993]. Testing for HCV antibodies and HCV RNA also revealed that persistent HCV infection can occur in patients without biochemical evidence of liver inflammation [Esteban et al., 1991; Alberti et al., 1992; Brillanti et al., 1993; Yuki et al., 1994a].

Asymptomatic HCV infection is frequent in haemodialysis patients. On average, the HCV seroprevalence in such patients is about 20% with first-generation HCV antibody assays, but a higher prevalence has been reported using more sensitive second-generation assays [Esteban et al., 1989; Jeffers et al., 1990; Vitale et al., 1993]. These HCV-seropositive haemodialysis patients are often symptom-free and have normal serum aminotransferase levels.

To gain insight into the development of asymptomatic HCV infection in this population, the occurrence of biochemical remission in the natural course of non-A, non-B (type C) hepatitis was studied in haemodialysis patients. The relation of biochemical remission of the disease to HCV replicative levels, which affect responses to antiviral therapy, was also investigated.

PATIENTS AND METHODS

Patients

Serological testing for HCV was carried out using a second-generation HCV antibody assay for 540 patients who were on chronic out-patient haemodialysis at Inoue Hospital, Osaka, Japan. HCV antibody was found in 125 (23%) patients. Serum alanine aminotransferase (ALT) levels were within the normal range (≤45 U/L) in 110 (88%) patients while the remaining 15 (12%) had elevated ALT levels. Of the 125 patients, 62 (50%) aged 20–77 yr (median 54) (27 males and 35 females) had received detailed follow-up for 9–218 mon (median 115) after the onset of non-A, non-B (type C) hepatitis, and were included in this study. The causes of their renal failure were chronic glomerulonephritis (n = 49), diabetic nephropathy (n = 6), polycystic kidney disease (n = 4) and obstructive nephropathy (n = 3).

Biochemical tests including serum ALT activity were

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carried out monthly after the diagnosis of renal disease. They were tested for hepatitis B surface antigen (HBsAg) every 1–3 mon. Since serological tests for HCV became available in 1990, HCV antibody was also examined every 3–6 mon. The onset of hepatitis C in these 62 patients occurred 6–108 mon (median 13) after their entry to the haemodialysis program. For >2 yr, they had repeatedly normal ALT values, but all had at least two abnormal values separated by one or more weeks. Hepatotropic viruses other than HCV and nonviral causes of hepatocellular injury were excluded by conventional clinical and laboratory studies. In 20 of the 62 patients, HCV antibody seroconversion was observed after the onset of the disease. The remaining 42 patients contracted non-A, non-B hepatitis before the discovery of HCV and were shown to have HCV infection when HCV antibody assays became available in 1990. All patients remained seropositive for HCV until the end of the follow-up. There was no cause of hepatocellular injury other than HCV during the entire follow-up period. These patients remained persistently negative for HBsAg and did not have a history of hepatotoxic drugs or alcohol abuse (>80 g/day) or any evidence of autoimmune liver disease.

Serological Testing

Serum samples were tested for HBsAg by radioimmunoassay (Abbott Laboratories, North Chicago, IL). For the diagnosis of non-A, non-B hepatitis, an enzyme immunoassay for IgM antibody to hepatitis B core antigen, a radioimmunoassay for IgM antibody to hepatitis A virus (Abbott Laboratories) and indirect immunofluorescence tests for antibodies to cytomegalovirus and Epstein-Barr virus were also carried out to exclude other types of viral hepatitis. HCV antibody was examined by a first-generation enzyme-linked immunosorbent assay or a second-generation radioimmunoassay (Ortho Diagnostic Systems Co., Ltd., Tokyo, Japan). The assays were done in duplicate. HCV antibody profiles were investigated by a second-generation recombinant immunoblot assay (RIBA-2) (Ortho Diagnostic Systems Co., Ltd.), which is capable of detecting antibodies to HCV core (C22-3), NS3 (C33C) and NS4 (5-1-1 and C100-3) proteins. The RIBA-2 test results were reported as "reactive" (reactivity with any two antigens), "indeterminate" (reactivity with just one antigen) or "non-reactive" (no reactive antigens). Sample reactivity to superoxide dismutase (SOD), to which all the HCV antigens are fused, was also assessed in the assay.

Detection and Quantification of Serum HCV RNA

Detection and quantification of serum HCV RNA were undertaken on serum samples drawn immediately before the haemodialysis procedure. Serum HCV RNA sequences were detected by reverse transcription and polymerase chain reaction (RT-PCR). HCV RNA was extracted from 100 μL of serum sample, copied into cDNA by reverse transcription, and amplified by PCR as described elsewhere [Hagiwara et al., 1993]. Primers were derived from the 5′-noncoding region of the published sequence [Takamizawa et al., 1991], which is highly conserved among HCV clones: antisense primer 5′ATGGTGCACGGTCTACGAGACCTCC3′ and sense primer 5′CACTCCCCTGTGAGGAACTACTGTC3′. The PCR mixtures were amplified in a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) for 40 cycles (94°C for 0.5 min; 55°C for 1 min; 72°C for 1 min), followed by a 10-min final extension at 72°C. A portion of the PCR products was fractionated by agarose gel electrophoresis, transferred onto a nylon membrane, hybridized to a ^{32}P-labelled HCV cDNA between the two primers, and autoradiographed. Because of the extreme sensitivity of PCR, great care was taken to prevent false-positive PCR results, and the contamination avoidance measures of Kwok and Higuchi [1989] were applied strictly throughout. Also, we included nine test samples, two negative control sera from healthy individuals without risk factors for HCV infection, and one sample of distilled water to prevent false-positive readings. The assay was repeated at least twice on each sample to confirm the reproducibility of the results.

Quantification of serum HCV RNA was done using a branched DNA (bDNA) assay (Chiron HCV-RNA, Chiron Corporation, Emeryville, CA) [Lau et al., 1993]. The assay was carried out according to the manufacturer's instructions. The number of light counts measured is directly proportional to the amount of HCV RNA equivalents present in sera. HCV RNA can be quantified by comparing the relative luminescence values obtained from each sample to the standard curve. Specimens with quantification values over the cut-off value of the kits (350,000 HCV RNA Eq/mL) were considered positive. All assays were done in duplicate, and the mean quantification value of the duplicates was calculated. The results were expressed as $\log_{10}$ (HCV RNA equivalent per mL). In preliminary experiments, the bDNA assay was compared with a competitive RT-PCR assay for quantifying serum HCV RNA [Hagiwara et al., 1993]. Thirty-nine serum samples, which had been shown to contain 10^4–$10^{9.5}$ copies of HCV RNA per mL serum (median 10^7) by the competitive RT-PCR assay, were measured by the bDNA assay. HCV RNA was detected in 37 (95%) of the 39 samples, and the titres ranged between $10^{5.6}$ and $10^{7.9}$ Eq/mL (median $10^{6.5}$). Two samples with low titres of HCV RNA (10^4 and $10^{4.5}$ copies/mL, respectively) were negative by the bDNA assay. In the former 37 samples, a significant correlation was found between quantification values from the competitive RT-PCR assay and those from the bDNA assay ($r = 0.72$, $P < 0.01$). An arbitrary value of 175,000 Eq/mL was attributed to the serum samples positive by RT-PCR but negative by bDNA assay. A value of 0 Eq/mL was attributed to the serum samples negative by RT-PCR.

Statistical Analysis

Statistical analysis for group comparisons was done by the χ^2 method and the Wilcoxon nonparametric test. Correlations between the variables were calculated us-

TABLE I. Serum Alanine Aminotransferase (ALT) Profiles of 62 Haemodialysis Patients After the Onset of Non-A, Non-B (Type C) Hepatitis

Characteristic	No. (%)
Peak ALT during the acute phase (× normal)[a]	
<2.5	25 (40%)
2.5–5	16 (26%)
>5	21 (34%)
Duration of illness (month)[b]	
<6	10 (16%)
6–24	14 (23%)
25–60	12 (19%)
61–120	11 (18%)
>120	15 (24%)
Months with normal ALT after the last episode of ALT elevation	
<6	13 (21%)
6–24	18 (29%)
25–60	16 (26%)
61–120	8 (13%)
>120	7 (11%)

[a]Values are multiples of the upper limit of normal.
[b]Duration of illness was defined as the time between the onset of acute hepatitis and the last episode of serum ALT elevation.

ing Spearman rank order correlations. A value of $P < 0.05$ (two-tailed) was considered to indicate significance.

RESULTS

The natural course of hepatitis C in haemodialysis patients was investigated in the 62 cases. At the end of follow-up, they were all symptom-free, and 57 (92%) patients showed normal serum ALT levels. With respect to the route of infection, serum ALT elevation occurred 1–6 mon after blood transfusion in 25 cases, and their illness was considered post-transfusion hepatitis C. The other 37 cases had no apparent risk factor for the onset of hepatitis, and their illness was considered sporadic hepatitis C. After the onset of the disease, the 62 patients were followed for 9–218 mon (median 115) with monthly tests for serum ALT levels (Table I). Their peak serum ALT levels ranged between 87 and 1,033 U/L (median 141). The duration of serum ALT fluctuation from the onset to the last episode of ALT elevation ranged between 1 and 206 mon (median 39). This fluctuation lasted for at least 6 mon in 52 (84%) patients. However, abnormality in serum ALT activity tended to ameliorate with time, and 57 (92%) of the 62 patients had a period of normal serum ALT lasting from 1 to 175 mon (median 31). Until the end of follow-up, normal levels of serum ALT were observed for more than 2 yr in 31 (50%) patients and for more than 5 yr in 15 (24%) patients, thus indicating biochemical remission of the disease.

These patients were persistently seropositive for HCV until the end of the follow-up, when HCV antibody profiles and HCV replicative status were investigated. Antibodies to C22-3, C33C, 5-1-1 and C100-3 were detected in 60 (97%), 54 (87%), 33 (53%) and 27 (44%) patients, respectively. According to the RIBA-2 results, 54 (87%) patients were considered reactive, and the remaining eight patients indeterminate. Of the latter eight patients, seven had antibody to C22-3 and one to C33C. None of the patients had antibody to SOD. Ongoing HCV replication was confirmed by RT-PCR for the detection of serum HCV RNA in 59 (95%) cases including seven of the eight indeterminate according to the RIBA-2 results. Serum HCV RNA was quantified further by the bDNA assay in the 59 patients with serum HCV RNA levels detectable by RT-PCR. Of these 59 cases, 46 (78%) patients had high viraemic levels detectable by the bDNA assay, and their serum HCV RNA levels ranged between $10^{5.6}$ and $10^{7.6}$ Eq/mL (median $10^{6.6}$). The remaining 13 patients had low viraemic levels below the bDNA cut-off.

HCV replicative states assessed by the concentration of serum HCV RNA were correlated with the patients' clinical course after the onset of hepatitis. The patients' serum HCV RNA levels had no relation with the time from the onset of hepatitis. As for the relation with serum ALT profiles during follow-up, HCV replicative levels did not correlate with the degree of serum ALT elevation during follow-up. However, a significant relationship was found between the duration of biochemical remission of the disease and HCV replicative levels at the end of follow-up. Serum HCV RNA levels were significantly lower in the 15 patients showing normal ALT levels for more than 5 yr (median RT-PCR+ve/bDNA-ve, range RT-PCR-ve to $10^{6.7}$ Eq/mL) than in the 47 cases with normal ALT levels for less than 5 yr (median $10^{6.6}$, range RT-PCR+ve/bDNA-ve to $10^{7.6}$ Eq/mL) ($P < 0.01$). Conversely, only seven (15%) of the 46 bDNA-positive patients had serum ALT normalization for more than 5 yr, which was less frequent compared with eight (50%) of the other 16 patients ($P < 0.01$). Moreover, a significant inverse relation was seen between serum HCV RNA levels at the end of follow-up and the duration of biochemical remission from the last episode of serum ALT elevation to the end of follow-up ($r = -0.46$, $P < 0.01$) (Fig. 1).

DISCUSSION

HCV infection is prevalent in haemodialysis units, and hepatitis C transmission is a serious health problem. HCV infection in haemodialysis patients is usually asymptomatic, and abnormality in serum aminotransferase activity is infrequent [Esteban et al., 1989; Jeffers et al., 1990; Vitale et al., 1993].

In the present study, the long-term biochemical course of hepatitis C in haemodialysis patients was investigated. Forty percent of the patients contracted post-transfusion infection while the exact mode of infection was unclear in the remaining 60% patients. These findings suggest that non-transfusion-associated spread of HCV may be frequent and unnoticed in haemodialysis patients. Serum aminotransferase levels fluctuated for at least 6 mon in more than 80% of the patients, indicating strongly the chronicity of the disease. However, biochemical disease activity tended to ameliorate with time. By the end of the follow-up, 50% patients had long-term biochemical remission of more than 2 yr, and more than 90% had normal aminotransferase activity at the end of follow-up. These findings indicate that in haemodialysis

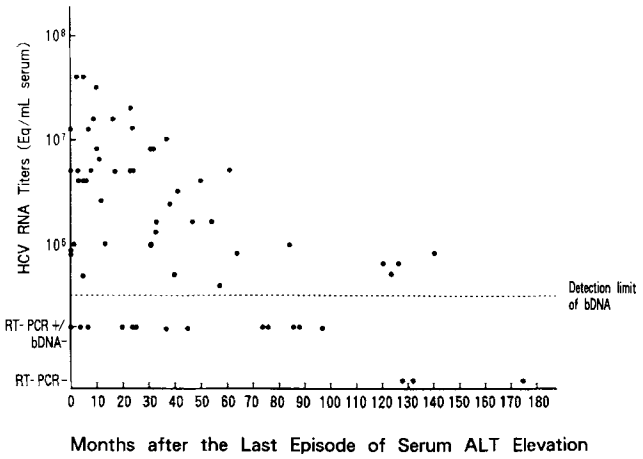

Fig. 1. Relationship between the duration of biochemical remission of non-A, non-B (type C) hepatitis and serum HCV RNA titers in haemodialysis patients ($r = -0.46$, $P < 0.01$).

patients, biochemical remission of hepatitis C may occur frequently during follow-up and contribute to the high prevalence of asymptomatic HCV infection in this population.

The present study revealed that HCV replication persists after biochemical remission in most cases. HCV replication was assessed further by the levels of serum HCV RNA. Recently, HCV replicative states have attracted considerable interest because they influence the responses to antiviral therapy and may be related to the infectivity of the disease. A wide range of HCV viraemic levels was observed in haemodialysis patients with asymptomatic HCV infection. The viraemic levels had an inverse correlation with the duration of biochemical remission and were low in patients showing long-term remission. These findings suggest that the biochemical remission of hepatitis C observed in haemodialysis patients may be associated with viral attenuation. Further studies are necessary to clarify the relative contributions of various host and virologic factors in different HCV replicative states. Understanding the importance of such factors is critical to the development of therapeutic strategies.

The current study showed that hepatitis C in haemodialysis patients may resolve itself frequently, at least biochemically, and result in an asymptomatic HCV carrier state with low viraemic levels. Thus far, several reports suggested that biochemical remission of hepatitis C can occur after long-term follow-up in patients not undergoing haemodialysis [Berman et al., 1979; Tremolada et al., 1988; Alter et al., 1992] although HCV viraemic levels were not investigated in relation to biochemical remission. To clarify further the nature of the biochemical and virological course of hepatitis C in haemodialysis patients, a control study in a comparable group of patients not undergoing haemodialysis seems necessary.

At present, controversy remains on the clinical management of asymptomatic HCV infection. Although detailed histological analysis was not available for our symptom-free haemodialysis patients, it has been reported that low viraemic levels in asymptomatic HCV carriers are associated with low levels of liver inflammation in biopsy specimens [Naito et al., 1994; Yuki et al., 1994b]. However, the stages of liver disease in such patients may be advanced and compatible with liver cirrhosis. Moreover, it remains unclear whether persistent, low-level viral replication with normal aminotransferase levels is associated with the progression of liver disease or a long-term nonprogressive HCV infection. To address these issues, further investigations are necessary to clarify the sequelae of the asymptomatic HCV carrier state and the effects on mortality.

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